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Discovery

Synthesis, Characterization and Antimicrobial Activity Studies of N'-(2-(3-Chloro-2-Oxo-Substitutedazetidin-1-Ylamino) Quinazolin-4-Yl) Isonicotinohydrazide

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ABSTRACT

In the present study we have synthesized N'-(2-(3-chloro-2-oxo-substitutedazetidin-1-ylamino) quinazolin-4-yl) isonicotinohydrazide using conventional methods and studied theirH^1 NMR and IR spectrum. We also studied antifungal and anti bacterial activities of the synthesized compound.

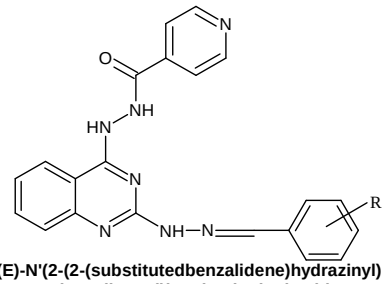
Keywords: Isoniazid, IR/NMR Spectroscopy, Antibacterial, Antifungal activity.

1. INTRODUCTION

Schiff base are condensation products of primary amine and aromatic aldehydes. They are known to exhibit potent antibacterial (Desai and Naik, 2004; Desai and Desai, 2005; Kumar et al., 2010), antifungal (Kumar and Dhakarey, 2003; Aliasghar et al., 2007), anticonvulsant, anti inflammatory activities. In addition some Schiff base show pharmacologically useful activities like anticancer (Nair et al., 2006), antihypertensive and hypnotic (Sridhar et al., 2002) activities. Azetidinones which are part of antibiotics structure are known to exhibit interesting biological activities. A large number of 3-chloro monocyclic β-lactam possesses powerful antibacterial, antimicrobial (Jarrahpour, 2004; Desai and Desai, 2005; Wadher, 2009), anti-inflammatory (Chikhaliya et al., 2006), anticonvulsant and antitubercular (Rajasekaran et al., 2010) activities. They also function as enzyme inhibitors and are effective on the central nervous system (Freddy and Mishra, 2004; Patel and Mehta, 2006; Chavan and Pai, 2007) they are the carbonyl derivatives of azetidines containing carbonyl group at the position-2. These are also known as 2-azetidinones or more commonly β-lactam. Azetidinones or β-lactam chemistry is of great importance because of the use of β-lactam derivatives as antibacterial agents (Kumar et al., 1983).

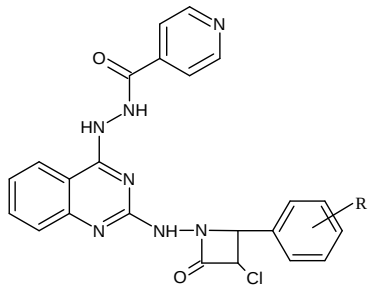
Cycloaddition of monochloroacetylchloride with imine (schiff base) result in formation of 2-azetidinone (β-lactam). The reaction involves direct acylation of imine with monochloroacetylchloride. The reaction is carried out with base as triethylamine gives β-lactam. Although variety of drugs have been developed for treating bacterial and fungal diseases, the basic difficulty experienced with these infections are the rapid development of drug résistance to the infectious strains. Review of literature reveals that 2-azetidinones are reported to possess significant antitubercular, antibacterial & antifungal activities. In view of these facts, synthesis of certain Azetidinone containing ciprofloxacin and isoniazid moiety has been undertaken in the hope of getting better bioactive agents. The constitution of all compounds synthesized was established by elemental analysis, IR and H1 NMR spectral study. Compounds were also evaluated for anti bacterial and anti fungal activities.

REACTION SCHEME



(E)-N'(2-(2-(substitutedbenzalidene)hydrazinyl) quinazolin-4-yl)isonicotinohydrazide

Tri ethyl amine
Chloro acetyl chloride
DMF
8-10hrs



N'-(2-(3-chloro-2-oxo-substitutedazetidin-1-ylamino) quinazolin-4-yl)isonicotinohydrazide

Where R =4-CH_3,3-CH_3-4-OH, 4-Cl, 2-NO_2, 2:4-Cl_2, 2-OCH_3, 4-NO_2, 4-H, 4-$N(CH_3)_2$,2OH

2. MATERIAL AND METHODS

All the chemicals used were of pure grade (Merck and B.D.H). The melting points of all compounds were determined by open capillary method and were uncorrected.

3. EXPERIMENTAL

Table 1

Characterization Table of N'-(2-(3-chloro-2-oxo-substitutedazetidin-1-ylamino) quinazolin-4-yl) isonicotinohydrazide

No	R	Molecular formula (M. wt.)	Yield (%) (per./ hrs.)	M.P. ^{0}C.
2a	4-CH_3	$C_{23}H_{17}ClN_8O_4$ (471.54)	82 (9)	186-87
2b	3-0CH_3,4-OH	$C_{24}H_{20}ClN_7O_4$ (505.92)	80 (10)	178-79
2c	2-NO_2	$C_{23}H_{17}ClN_8O_4$ (504.89)	69 (9)	225-26
2d	4-Cl	$C_{23}H_{17}Cl2N_7O_2$ (494.34)	74 (9.5)	191-92
2e	2,4-$(Cl)_2$	$C_{23}H_{16}Cl_3N_7O_2$ (528.78)	75 (9.5)	203-04
2f	2-OCH_3	$C_{24}H_{20}ClN_7O_3$ (489.92)	80 (8.5)	165-66
2g	4-NO_2	$C_{23}H_{17}ClN_8O_4$ (504.89)	56 (9)	201-202
2h	4-H	$C_{23}H_{18}ClN_7O_2$ (459.89)	66 (8)	181-82
2i	4-$N(CH_3)_2$	$C_{25}H_{23}ClN_8O_2$ (502.96)	78 (10)	170-71
2j	2-OH	$C_{23}H_{18}ClN_7O_3$ (475.89)	72 (8)	155-156

Table 2

Signal positio(δ ppm)	Inference
8.62	-NH (a)
9.39	-NH(b)
6.01	-NH(c)
4.56	-NH_2
5.25	-CH
5.52	-CH-Cl
5.97 to 7.89	Aromatic Protons

Preparation of 2, 4-Quinazolinedione

In a 1000ml beaker, a mixture of (20 gm, 0.146mole) of anthranilic acid, 700ml of warm water (40^0 c) and glacial acetic acid (11ml, 0.19mole) was stirred mechanically and allowed to cool to room temperature. A freshly prepared solution of (15gm, 0.185mole) of potassium cyanate in 50ml of water was then added drop wise with stirring over a period of 15 to 20 minutes. The resulting pasty mixture was stirred for 20 minutes and then (200gm, 5mole) of Sodium hydroxide was added slowly in small portion. During this addition the reaction mixture was kept below 40^0 c by cooling in a cold water bath. A clear solution was obtained momentarily, but in a sort time a fine granular precipitate of the hydrated mono sodium salt of benzoylene urea precipitate. After the mixture has cooled over night in an ice box, the precipitated sodium salt was collected on a Buckner funnel. The colorless salt was dissolved in 1 lit of hot water and the solution was filtered. The compound was precipitated by adding dilute sulfuric acid (1:1) with vigorous stirring until the liquor was acidic to litmus the material was collected, washed with water and dried in an oven at 100 ^{0}C.

Preparation of 2, 4-Dichloroquinazoline

In a 250 ml R.B.F., a mixture of (20gm, 0.123mole) of 2, 4-quinazolinedione, 200ml of phosphorus oxychloride and of tri-n- propyl amine (59gm 0.226mole) refluxe for 30minutes to yield a clear solution and volatile liquid was removed by distillation. The remaining mass was added in to ice and isopropyl alcohol. The product was extracted from residue with (4x200 ml) portion of hot n-heptane containing 2% tri-n-propyl amine. The combine extracts, at room temperature was diluted with enough benzene to dissolved crystallized solid. The organic solution was washed with 400ml of 5% NaOH and three times with water. The solvent was removed in vacuo, and the residual solid was recrystallized from 2:1 ethyl acetate: n-heptane to give 12.1 gm. of 2,4-dichloroquinqzoline as white needles. A second crop of product was obtained by reducing the mother liquor to one fourth the volume to give 6.7 gm. The combine yield of the two crops was 18.8 gm.

Preparation of N'-(2-chloroquinazolin-4-yl)isonicotinohydrazide

In 250 ml R.B.F., a solution of 2,4-dichloroquinazolin(0.01 mole) dry ether(20 ml) was taken & added Isoniazid (0.01 mole) the mixture was stirred at room temperature for 24 hr, the ether was removed on a steam bath & the residue dissolved in approximately 150 ml of water. The Solution was made basic with 20 % aqueous NaOH & Extracted three times with chloroform. The Combined Chloroform Extract were washed once with water, once with saturated Sodium Chloride solution & then dried over magnesium sulphate. After removal of chloroform the residue was purified by recrystallization using Ethanol.

Preparation of N'-(2-hydrazinoquinazolin-4-yl)isonicotinohydrazide

In a 250 ml R.B.F.,solution of N'-(2-chloroquinazolin-4-yl)isonicotinohydrazide. (0.01 mole) in DMF was taken & added Hydrazine hydrate (80%, 0.01 mole). The reaction Mixture was Stirred at 110 ^{0} C for 18 hrs. The resulting mixture was cooled to room temperature, neutralized with ammonia. The Solid was filtered, washed, Dried & Recrystallised using ethanol.

Preparation of (E)-N'-(2-(2-(substituted benzalidene) hydrazinyl) quinazolin-4-yl) isonicotino hydrazide

In a 250 ml R.B.F., N'-(2-hydrazinoquinazolin-4-yl)isonicotinohydrazide (3.10 gm ,0.01 mole) in THF was taken & Substituted benzaldehyde(0.01 mole) and 1 drop of conc. H_2SO_4 were added & refluxed for 9 – 10 hrs. The completion of reaction was monitored by TLC Examination 1:1 (Hexane: ethyl acetate). After completing of reaction the flask was cooled over night and residue was filtered off. The solid thus separated was filtered, washed with water & recrystalised from ethanol.

Table 3

Adsorption	2a	2c
N-H (st)	3425.92	3255.38
-CH_3	2957.30	--------
-Cl	---------	762.74
-C=O	1675.35	1630.58
-C=N(st)Quinazoline	1602.07	1611.30
N-C-N (st)Quinazoline	1368.72	1382.76
Ar (C=C)	1556.27	1515.83
In plane Ar-H	1034.14	1092.52
Ar-H (b) Vib.	840.81	837.95
Out plane Ar-H	668.69	668.24

Preparation of N'-(2-(3-chloro-2-oxo-substitutedazetidin-1-ylamino) quinazolin-4-yl) isonicotinohydrazide

In a 250 ml R.B.F, (E)-N'-(2-(2-(substitutedbenzalidene) hydrazinyl) quinazolin-4-yl) isonicotino hydrazide (0.01 mole) and triethylamine (0.03 mole) were dissolved in DMF (30 ml). Chloroacetyl chloride (0.012 mole) in DMF was added drop wise at 0-5 ºC. The reaction mixture was stirred for 2 hr at room temperature and refluxed for 8-10 hr. Excess of solvent was then removed by distillation and cooled. The solid thus separated filtered, washed, dried and recrystallised from glacial acetic acid.

4. RESULTS AND DISCUSSION

All the tested compounds have shown (Table 1) antibacterial activity to some extent. Among the tested compounds 2a and **2j** showed very good activity against the tested organisms. Compounds **2c**, **2d**, **2e** and **2g** are moderate antibacterial activity. The compounds **2c**, and **2i** showed good antifungal activity. All the compounds synthesized possess electron releasing groups, on both the aromatic rings. Therefore from the results it is evident that compounds having electron releasing groups like methyl, hydroxy and methoxy may be responsible for antibacterial and antifungal activities.

Table 4

Antimicrobial activity of compound

Code No.	*S. aureus*	*P.aeruginosa*	*E. coli*	*S. pyogenus*
2a	100	200	100	62.5
2b	100	500	500	100
2c	125	200	62.5	200
2d	125	200	200	250
2e	100	250	125	250
2f	125	500	250	500
2g	200	200	100	200
2h	200	250	200	125
2i	250	250	100	200
2j	100	100	200	100
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50

^{1}H NMR Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is one of the latest physical methods of investigating organic compounds. The scale of the spectrum is usually marked in parts per million (ppm) of the applied field or in frequency units (Hz). ^{1}H-NMR spectra were recorded on Bruker WM 400FT MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference. The data of compound (4a) is summarized in Table 2.

Infrared spectra

The systematic interpretation of the infra - red spectrum is based upon the empirical data obtained by assigning infra-red absorption values to the structural units a characteristic of different structural units. Infra - red spectra were recorded in KBr on a Shimadzu FTIR spectrophotometer. The data of the structure is summarized in Table 3.

Table 5
Antifungal activity of compound

Code No	*C.albicans*	*A.niger*	*A.clavatus*
2a	500	1000	500
2b	100	500	500
2c	500	100	100
2d	125	500	125
2e	250	125	250
2f	1000	100	1000
2g	1000	500	500
2h	500	250	1000
2i	250	100	100
2j	1000	250	125
Nystatin	100	100	100
Greseofulvin	500	100	100

Antimicrobial activity

The examination of antimicrobial activity of organic compound and its all substitution reveals that the compound is moderately more or less active against various organisms. The synthesized compounds were screened for their antibacterial activity using *S.aureus, E. coli, P.aeruginosa and S. pyogenus* (Table 4). Control experiment was carried out under similar condition by using ampicillin and chloremphenicol as standard. The inhibition zone measure in mm showed that compound **2a** and **2j** were more active than other compounds tested against the above microbes. Anti-bacterial activity of Compounds was investigated via the broth dilution method (Thornsberry and Stalon, 1975; Radetsky et al., 1986; Carson et al., 1995).

Anti-fungal activity

The investigation of antifungal activity of Compound4a-4j was carried out with the stiff plate agar diffusion method (Leach et al., 2000) against *C.albicans,A.niger and A.clavatus.* The amount of microbial cells was 109c.f.u. /ml. Incubation period was 24 h at 35 °C for bacteria. Antibiotics nystatin and greseofulvin were used as standards. The bacterial cultures, standards, and obtained substances in 5 mg/ml concentration were streaked across grooves and then allowed to dif-fuse in the agar nutrient plate (Table 5).

5. CONCLUSION

The work has approached towards the synthetic and biological approach of these wonder molecules. Anti-bacterial property of the synthesized compounds has exhibited very good inhibition; the compounds 2a and 2j have exhibited outstanding activity towards *S.aureus, E. coli, P.aeruginosa and S. pyogenus.* Compound **2c** shows good activity against E.Coli. compoound **2a** shows good activity against *S. pyogenus.* But the systematic substitution at various position and other derived compounds have shown remarkable antifungal properties. The compounds **2c** and **2i** have exhibited good activity towards *A.niger, A.clavatus* and C. *albicans.* Compound **2d** shows good activity against *A.clavatus* and C. *albicans* the remaining compounds have shown poor antifungal activity indicating less biological importance for a synthetic chemist.

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